Aus dem Röntgeninstitut der Universität Bern

(Direktor: Prof. Dr. A. Zuppinger)

# CHOLINESTERASE ACTIVITY UNDER THE INFLUENCE OF HISTAMINE, POTASSIUM CHLORIDE, AND ROENTGEN THERAPY

INAUGURAL-DISSERTATION

zur
Erlangung der Doktorwürde
der Medizinischen Fakultät
der Universität Bern

vorgelegt von

Paul Hunter

von New York, N.Y., U.S.A.

ARNAUD DRUCK BERN 1956

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# INTRODUCTION

With the increased use of Roentgen therapy the advent of radiation sickness and its prevention or treatment has assumed ever increasing importance. The supposed intimate relationship of cholinesterase to radiation sickness (1) provoked our interest to a study of histamine as a possible inhibitor and potassium chloride as a possible activator of the enzyme (3, 14).

The observations of the cholinesterase activity of human serum during roentgen ray therapy were undertaken with the anticipation that the titrimetric method might prove appropriate for indicating the approach of dangerously low cholinesterase levels during roentgen treatment or among personnal exposed to roentgen rays. It was thought that a reliable test might be worked out involving the cholinesterase level that would give information as to the status of the erythropoetic organs sooner and more exactly than the white blood cell counts or bone marrow examinations now in use.

It is generally thought that radiation sickness is in part a manifestation of a cholinergic state (4) which parallels a fall in the normal cholinesterase of the blood. This decrease allows a predominating influence of acetylcholin with the result of a longer and more pronounced stimulation of the parasympathetic nervous system. While the clinical picture of radiation sickness may vary depending on the roentgen dosage, time, and area of exposure; the general symptoms of nausea, vomiting, varying blood pressure, diarrhea, sphincter spasm and circulatory collapse can be explained on a basis of the cholinergic condition.

The problem of cholinesterase inhibitors is of paramount interest from a clinical standpoint. To establish that histamine or potassium chloride exerted a significant influence on the cholinesterase activity would immediately provide a route to aetiologic therapy. Bayer and Wense (2) have maintained that the inhibiting factor is histamine. Our investigations on mouse serum, both in vitro and in vivo have failed to substantiate their results. That potassium chloride favors the cholinesterase activity was indicated by Mendel and Rudney (14). In this case too our results with physiologic concentrations of potassium chloride have not shown that substance to have any effect on the cholinesterase activity. These will be considered more fully in the discussion section of this paper.

To chart the effect of roentgen therapy on an individual with a test similiar to the titrimetric method employed here would provide a control over therapy that is at the present time lacking. Because of the general systemic nature of the cholinergic state, a charting of its degree, i.e. the cholinesterase level, would give us an index of the deleterious effects of the therapy on the organism as a whole. In

addition, since the bone marrow is repoted to be one of the building places of cholinesterase (5), a measure of its activity should give a simpler and possibly earlier criterion of the functioning status of the marrow than the cytologic examination now in use.

Our study of histamin as an inhibitor of cholinesterase is in essence an augmentation of the work of Bayer and Wense (2). They sensitized the leech with histamin and then took as a measure of the cholinesterase inhibition, the increased effect of acetylcholin on the back muscles. We have used a direct method to determine the cholinesterase working by measuring the hydrolysis of acetylcholin. This was done in vitro with mouse serum in direct mixture with high concentrations of histamine and subsequently with physiologic concentrations of histamine, Tables 1 and 2. Histamine was further investigated by injecting it into the tail veins of mice and then after various reaction times, measuring the cholinesterase activity of the serum. The concentrations used here were about ten times t e physiologic.

The potassium chloride effect was also studied in vitro. In this case too, physiologic concentrations of the salt were mixed with mouse serum and the cholinesterase activity measured by titrimetry.

In the study of the human subject undergoing roentgen therapy, the cholinesterase activity of the serum of cancer patients was determined before they had received any radiation treatment. Then at periodic intervals, (two to three days) during roentgen therapy, blood samples were taken and the cholinesterase activity determined. A control was attempted by periodically examining the serum of cancer patients who were not undergoing any radiation.

## METHOD (6)

### THE DETERMINATION OF CHOLINESTERASE ACTIVITY

A titration method was employed in which acetic acid from the hydrolysis of standard acetylcholin substrate solution was determined. The method introduced by Michel (7) was investigated by Lüthy (6) in his first orientating experiments. It was not found appropriate for our objectives in that the addition of a barbital buffer slowed the hydrolysis to such an extent that the measurement of weak inhibiting effects in vitro was not possible. The method here employed was based on that of Glick (8), Sanz (9) and Radouco and Frommel (10) with a few technical variations. The acid equivalents liberated by the hydrolysis were neutralized by the addition of 0,01N. sodium hydroxide from a microburette. In this way the pH was

maintained between very narrow limits. For each amount of alkali (recorded in cubic centimeters) added, the time necessary for the solution to return to its initial pH was recorded to the second. With an experimental time of 6 to 8 minutes a straight line graph was plotted, c.c. NaOH against Time in minutes. The slope of this graph is a measure of the hydrolysing activity of the cholinesterase. This value was corrected by subtracting the slope of a blank run, which represented the non-fermentative autolysis of acetylcholin, Fig. 1.

## EXPERIMENTAL MATERIAL

For the experiments in vitro on the histamin and potassium chloride; serum mixtures from 15 female, old (1 year) white mice were used. Mixtures were used in order to achieve a "normal" or "standard" serum solution with which experiments with, and without, inhibitor could be compared. Because of the wide variation of the cholinesterase activity of individual mice (6) such a comparison would have been very difficult if individual serums for individual experiments had been used. Obviously our term "normal" does not refer to or indicate a criterion for mouse serum but is only an arbitrary norm set up for the experiment. In the case of the in vivo experiments this standardization was not possible. This will be further considered in the discussion section of this paper.

With the in vivo experiments, the histamin was given intra-venously, via the tail veins. In all cases the serum was obtained by decapitating the mice and collecting the blood directly in centrifuge tubes. The blood was then centrifuged at 3000 r.p.m. for 15 minutes and the resulting clear supernatant serum pippeted off and stored until use under refrigeration. For each experiment 0,05 c.c. of the serum was mixed with 0,45 c.c. of 0,9 % NaCl, a dilution of 1:10. This represented the standard experimental serum solution. To this was then added 4,50 c.c. of an 11 millimole solution of acetylcholin. The liberated acetic acid was then determined by successively titrating to a constant pH of 7 and recording the time and amount of alkali added. A combination glass-calomel electrode in conjunction with a pH Meter (EA 125, Metrohm Herisau) served as a constant orientation as to the pH of the reacting solutions, Fig. 2. When histamin or potassium chloride were used the concentrations were calculated on a basis of the 0,05 c.c. serum and diluted with the 0,9 % NaCl. The mixture, inhibitor - Na-Cl, was then used to dilute the serum. In the case of the experiments with human serum a dilution of the serum of 50 % with 0,9 % NaCl gave optimal graphic results, Table 5. All determinations were made at a constant temperature of 37° C.

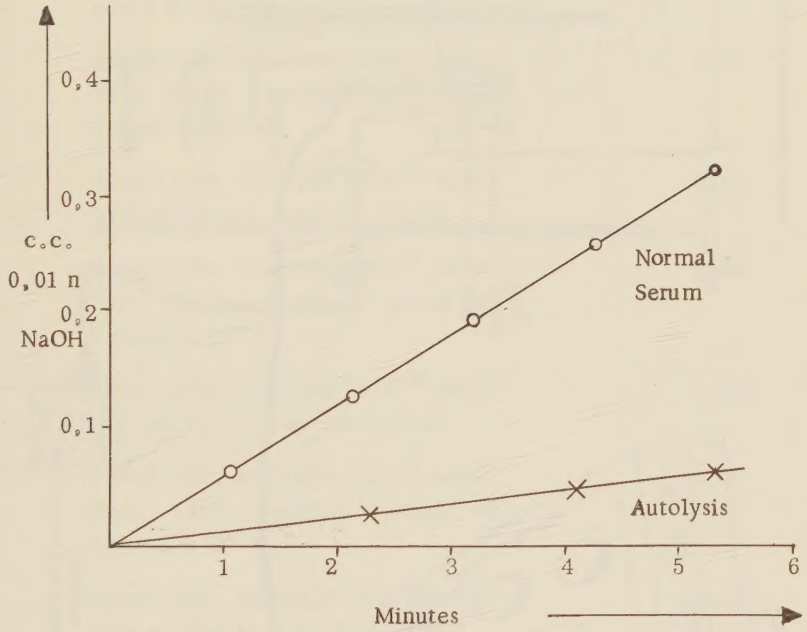


Fig. 1: Graph of Titration of Normal Serum and graph of non-fermentative autolysis of acetylcholin

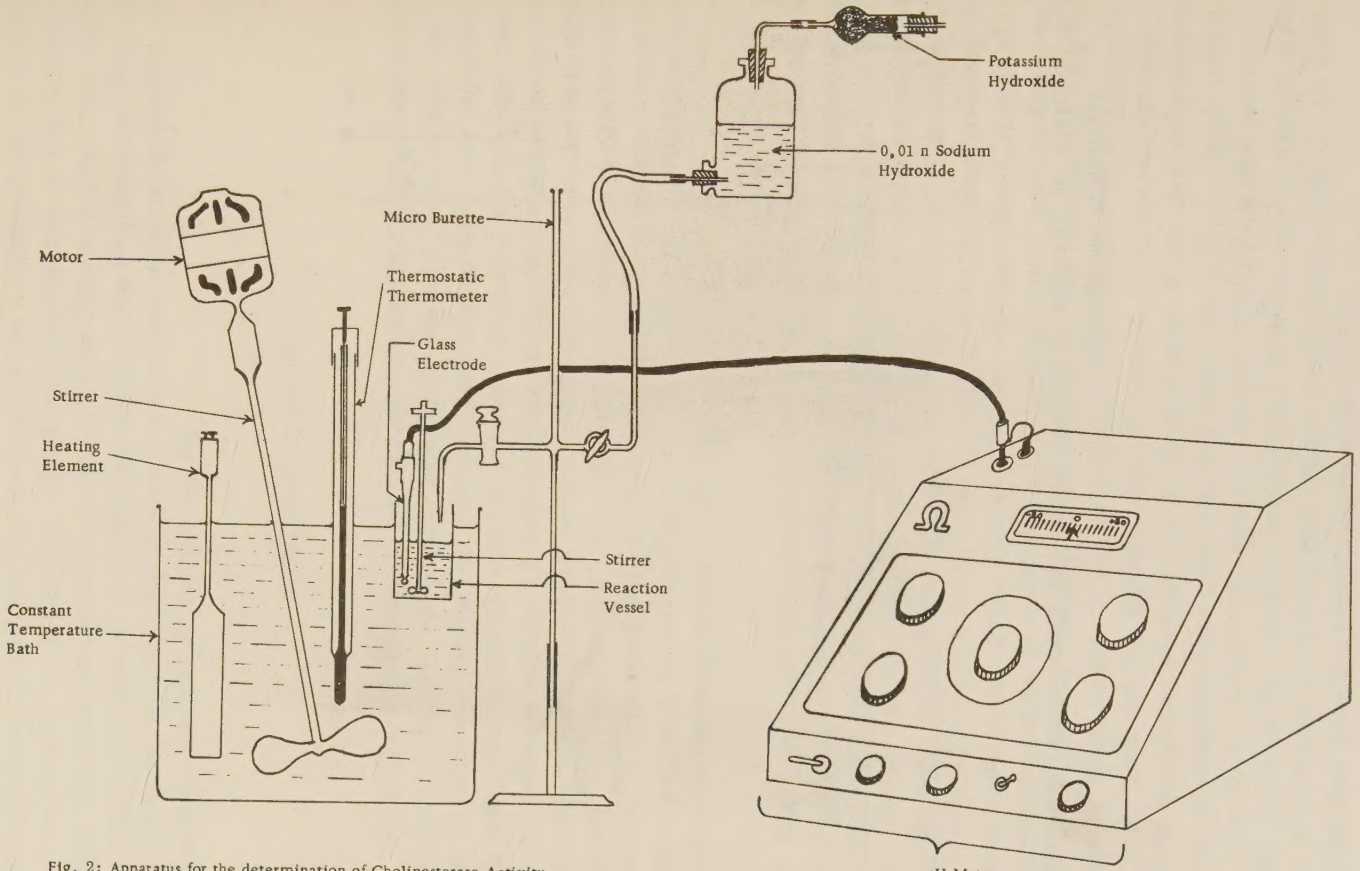


Fig. 2: Apparatus for the determination of Cholinesterase Activity

Table 1

Experiments with Histamin concentrations far above physiologic conditions

| Exp. | Conditions | Cholinesterase (++) Activity |
|---|---|---|
| 1. | Normal mouse serum without Histamin. | 0,58 |
| 2. | Serum plus $5x10^{-4}$ mole/liter Histamin without reaction period*. | 0,575 |
| 3. | Serum plus Histamin $5x10^{-4}$ mole/liter with reaction period of 2 hours at 37° C. | 0,60 |
| 4. | Serum plus Histamin $5x10^{-2}$ mole/liter without reaction period. | 0,47 |
| 5. | Serum plus Histamin $5x10^{-2}$ mole/liter with reaction period of 2 hours at 37° C. | 0,46 |
| 6. | Serum plus Histamin $5x10^{-3}$ mole/liter without reaction period. | 0,485 |
| 7. | Serum plus Histamin $5x10^{-3}$ mole/liter with reaction period of 2 hours at 37° C. | 0,36 |
| 8. | Serum plus Histamin $1x10^{-3}$ mole/liter without reaction period. | 0,48 |

* "reaction period" indicates a period of time during which the substrate mixture was allowed to react before the determination of the cholinesterase was undertaken.

(++) Cholinesterase activity is here expressed as the slope of the graph "c.c. NaOH used against Time in minutes", see page 5.

Table 2

Experiments with Histamin concentrations simulating physiologic conditions
Histamin concentrations here expressed in milligram per cent
In vitro experiments

| Exp. | Conditions | Cholinesterase Activity |
|---|---|---|
| 1m | Serum plus Histamin $5x10^{-5}$ | 0,56 |
| 2m | Serum plus Histamin $1x10^{-5}$ | 0,57 |
| 3m | Serum plus Histamin $5x10^{-6}$ | 0,55 |
| 4m | Serum plus Histamin $1x10^{-6}$ | 0,56 |
| 5m | Serum plus Histamin $5x10^{-7}$ | 0,58 |
| 6m | Serum plus Histamin $1x10^{-7}$ | 0,58 |

Table 3

In vivo experiments with histamin concentrations simulating physiologic conditions
Histamin concentrations expressed here in milligram per cent

| Exp. | Conditions | Cholinesterase Activity |
|---|---|---|
| 1i | Histamin $1x10^{-5}$ I.V. reaction period 15 minutes before decapitation. | 0,34 |
| 2i | " | 0,675 |
| 3i | " | 0,45 |
| 4i | " | 0,405 |
| 5i | " | 0,53 |
| 6i | " | 0,57 |
| 7i | " | 0,565 |
| 8i | Histamin $1x10^{-5}$ I.V. reaction period one hour before decapitation. | 0,675 |
| 9i | " | 0,35 |
| 10i | " | 0,58 |
| 11i | " | 0,54 |
| 12i | Histamin $1x10^{-5}$ I.V. reaction period two hours before decapitation | 0,585 |
| 13i | " | 0,555 |
| 14i | " | 0,44 |

Table 4

In vitro experiments on mouse serum with potassium chloride in concentration simulating physiologic conditions

Concentrations here expressed in milligram per cent

| Exp. | Conditions | Cholinesterase Activity |
|---|---|---|
| 1k | Serum plus 30 mg % KCl | 0,45 |
| 2k | Serum plus 60 mg % KCl | 0,47 |
| 3k | Serum plus 90 mg % KCl | 0,48 |
| Determination on "normal" serum for exp. 1k, 2k, & 3k | Normal mouse serum mixture without KCl | 0,48 |
| 4k | Serum plus 20 mg % KCl | 0,48 |
| 5k | Serum plus 15 mg % KCl | 0,49 |
| 6k | Serum plus 10 mg % KCl | 0,495 |
| 7k | Serum plus 5 mg % KCl | 0,50 |
| Determination on "normal" serum for exp. 4k,5k,6k, & 7k | Normal mouse serum mixture wihtout KCl | 0,50 |

Table 5

Experiments to determine the cholinesterase activity of human serum in various concentrations

| Exp. | Conditions | Cholinesterase Activity |
|---|---|---|
| 1s | Serum: 0,9 % NaCl = 1: 10 | 0,06 |
| 2s | " " = 1: 5 | 0,18 |
| 3s | " " = 1: 1 | 0,54 |
| 4s | Pure serum without thinning | 1,10 |

Table 6

Experiment to chart the cholinesterase activity of human serum in patients undergoing Roentgen therapy

| Exp. | Date of Blood taking | Cholinesterase Activity | Radiation (Air dose) | Diagnosis |
|---|---|---|---|---|
| [illegible] | 25 Aug. '53 | 0,55 | Before treat. | Squamose cell Ca. Left lung. |
| [illegible] | 28 Aug. '53 | 0,535 | 1200 r | |
| [illegible] | 1 Sept. '53 | 0,395 | 3600 r | Age: 54 |
| [illegible] | 3 Sept. '53 | 0,363 | 6800 r | Exitus: 6 Sept. '53 |
| [illegible] | 26 Aug. '53 | 0,665 | Before treat. | Lymphoid cell reticulo sarc. of the lung. |
| [illegible] | 1 Sept. '53 | 0,565 | 90 r | |
| [illegible] | 3 Sept. '53 | 0,49 | 120 r | |
| [illegible] | 5 Sept. '53 | 0,535 | 250 r | Age: 64 |
| [illegible] | 7 Sept. '53 | 0,515 | 310 r | |
| [illegible] | 18 Sept. '53 | 0,42 | 860 r | |
| [illegible] | 22 Sept. '53 | 0,47 | 1310 r | |
| [illegible] | 25 Sept. '53 | 0,42 | 1760 r | Exitus: 8 Nov. '53 |
| [illegible] | 3 Sept. '53 | 0,795 | Before treat. | Squamous cell Ca. left main bronchus |
| [illegible] | 7 Sept. '53 | 0,605 | 1200 r | |
| [illegible] | 9 Sept. '53 | 0,48 | 3600 r | |
| [illegible] | 11 Sept. '53 | 0,36 | 4800 r | Age: 72 |
| [illegible] | 14 Sept. '53 | 0,32 | 6000 r | |
| [illegible] | 18 Sept. '53 | 0,30 | 9600 r | |
| [illegible] | 22 Sept. '53 | 0,28 | 12600 r | |
| [illegible] | 25 Sept. '53 | 0,27 | 16200 r | Exitus: 9 Dec. '53 |

Table 6 (cont.)

| Exp. | Date of Blood taking | Cholinesterase Activity | Radiation (Air dose) | Diagnosis |
|---|---|---|---|---|
| 1Ma | 1 Sept.'53 | 0,91 | Before treat. | Recidivation in operation scar for Ca. solidum simplex of the mama. |
| 2Ma | 3 Sept.'53 | 1,055 | 500 r | |
| 3Ma | 5 Sept.'53 | 1,055 | 1000 r | |
| 4Ma | 7 Sept.'53 | 0,955 | 1250 r | |
| 5Ma | 9 Sept.'53 | 0,975 | 1750 r | Age: 75 |
| 6Ma | 11 Sept.'53 | 1,000 | 2250 r | |
| 7Ma | 18 Sept.'53 | 0,965 | 3750 r | |
| 8Ma | 22 Sept.'53 | 0,96 | 4550 r | Discharged as arrested. |
| 9Ma | 25 Sept.'53 | 0,975 | 4750 r | |
| 1Br | 9 Sept.'53 | 0,805 | Before treat | Struma maligna operation. Supra clavicular metastases |
| 2Br | 11 Sept.'53 | 0,975 | 600 r | |
| 3Br | 14 Sept.'53 | 0,84 | 1200 r | |
| 4Br | 16 Sept.'53 | 0,955 | 2000 r | |
| 5Br | 18 Sept.'53 | 0,98 | 2800 r | |
| 6Br | 22 Sept.'53 | 0,89 | 3800 r | |
| 7Br | 25 Sept.'53 | 0,87 | 5000 r | |

Table 7

Experiments to chart the cholinesterase activity of human serum in patients with cancer, but not undergoing roentgen therapy

| Exp. | Date of Blood taking | Cholinesterase Activity | Diagnosis |
|---|---|---|---|
| 1de | 28 Sept.'53 | 0,37 | Bronchial carc. with widespread metastasis. Age: 56 Exitus: 17 Oct.'53 |
| 2de | 30 Sept.'53 | 0,17 | |
| 3de | 2 Oct.'53 | 0,105 | |
| 1Sa | 25 Sept.'53 | 0,24 | Bladder carcinome |
| 2Sa | 28 Sept.'53 | 0,37 | Carc. cylindro cell. of the Antrum Ventriculi. Age: 73 Exitus: 13 Oct.'53 |
| 3Sa | 30 Sept.'53 | 0,31 | |
| 4Sa | 3 Oct.'53 | 0,26 | |
| 1Be | 25 Sept.'53 | 0,40 | Cancroid of the tongue with liver metastasis. Age: 80 Exitus: 19 Oct.'53 |
| 2Be | 28 Sept.'53 | 0,395 | |
| 3Be | 30 Sept.'53 | 0,21 | |
| 4Be | 2 Oct.'53 | 0,33 | |

# DISCUSSION OF HISTAMINE EFFECT ON SERUM CHOLINESTERASE

As stated previously, Bayer and Wense indicated that histamine acted as an inhibitor of cholinesterase (2). They sensitized the back muscles of the leech with histamine and then took as a measure of the cholinesterase inhibition, the increased effect of acetylcholin on these muscles. This indirect method seems open to criticism in that the contractility of a cell is not essentially a factor of the cholinesterase present but rather the sensitivity of the cell to acetylcholin. It seems quite possible that the histamine could increase the sensitivity of the cell to acetylcholin, giving an increased response, without actually influencing the cholinesterase in any particular.

The titrimetric method gives a more direct and absolute analysis in that it measures an actual hydrolysis of the acetylcholin.

Our initial experiments with histamine were carried out with rather high concentrations i.e. $5 \times 10^{-2}$ to $5 \times 10^{-4}$ moles per liter. Even though these concentrations were far beyond what might be expected under physiologic conditions they showed no variation in the cholinesterase working that could be attributed to a histamine inhibition. The slight decrease that was measured, experiments 4 - 8 Table 1, was thought due to the large amounts of alkali needed to initially neutralize the reacting solution. This decrease could possibly be explained on a basis of Augustinnsson's "Inhibition as a function of substrate concentration" (3) in which he showed cholinesterase inhibition following a bell curve with increased substrate concentration.

Further experiments were made with histamine in concentrations approximating those to be expected under physiologic conditions, i.e. $5 \times 10^{-5}$ to $5 \times 10^{-7}$, Table 2. Here it was not necessary to use large amounts of alkali to neutralize the reacting solutions. The results in this series showed practically no variation from the activity of the standard mouse serum solution.

The histamine experiments in vivo were made by injecting concentrations of histamine approximately 10 times the physiologic in the tail veins of mice. Various time periods from 15 minutes to 2 hours were allowed for the histamin to exert an effect, then the mice were decapitated and the serum cholinesterase activity determined in the usual manner. In these experiments it was not possible to use a "standard" mouse serum mixture because of the in vivo nature of the experiments. The results, Table 3 Exp. 1i to 14i show the same variations that could be expected from mice that had not been treated with histamine, Lüthy (6).

## DISCUSSION OF THE EFFECT OF POTASSIUM CHLORIDE ON CHOLINESTERASE

Mendel and Rudney (14) studied the effect of various salts on cholinesterase. They used "true cholinesterase", i.e. cholinesterase extracted from tissue (mouse brain) rather than serum or pseudo-cholinesterase. They found that with rather high concentrations (0,16 - 0,04 M.) that potassium chloride enhanced the effect of cholinesterase. They used the Warburg method and although they added Sodium bicarbonate to the reaction mixture their experiments are capable of being compared with these. However we were unable to detect any effect of potassium chloride on the activity of cholinesterase, Table 4. This might be due to the fact that the concentrations we used approximated physiologic limits whereas those used by Mendel and Rudney were far beyond the physiologic.

## DISCUSSION OF THE CHOLINESTERASE LEVEL IN CANCER PATIENTS

It was thought that the cholinesterase level of cancer patients receiving roentgen therapy would be significantly different than that of cancer patients receiving no radiation. Therefore a possible method could be devised to chart the effect of the treatment. Our investigations however have shown no significant difference in the cholinesterase level of cancer patients receiving radiation and those receiving none, Tables 6 and 7. The results do show however that there are quite marked fluctuations in the cholinesterase level of cancer patients. In general this agrees with the results of Strehler and Meyer (11) who found rather large fluctuations in a great variety of different diseases. These investigators, however, were unable to establish a variation that they thought was solely due to a malignome. Schmidt (12) however, using the Arburg method of determination, found significant decreases in the cholinesterase activity of 78 % of 67 cancer patients. Schmidt also noted that the cholinesterase returned to a normal level after surgical removal of the tumor. This offers a tempting conjecture when it is noted that two of our cases, Ma and Br Table 6, who showed increases in the cholinesterase level with the advent of therapy are still surviving six months later whereas the other patients, who showed great and progressive reductions in the cholinesterase activity, died in a much shorter time.

From these results it seems that this method is not appropriate to the charting of the effect of roentgen therapy. Strehler and Meyer (11) with reference to the work of Kraupp and Leonhartsberger (13) point out that the cholinesterase activity seems to

be intimately associated with the albumin synthesis and varies directly with the albumin content of the blood. While radiation doubtlessly effects the cholinesterase in lower animals, Lüthy (6), and probably effects it in man, Barnard (1); its close relationship with the blood protein system and the considerable variations in this system in pathological states probably far outshadow the effects of therapeutic radiation on the cholinesterase activity. Thus a cholinesterase determination as a measure of roentgen effect in a pathological condition would seem rather inconsequential.

## SUMMARY

The question as to whether the inhibition of cholinesterase resulting from roentgen radiation, is due to a direct effect of the radiation or due to a liberation of histamine or potassium chloride (14, 15) was studied.

The effect of histamine on mouse serum cholinesterase (pseudo-cholinesterase) was studied by means of the titrimetric method utilizing the glass-calomel electrode. Histamine concentrations both physiologic and above the physiologic range and in vitro as well as in vivo were examined. In no case, under any of these conditions, could a significant effect on the serum cholinesterase be determined.

The effect of potassium chloride on mouse serum cholinesterase was also studied with concentrations both in and above the physiologic range. These experiments were carried out in vitro. No effect of potassium chloride on the serum cholinesterase could be determined.

A study of the cholinesterase activity of humans undergoing roentgen therapy was undertaken. According to this study, a determination of the serum cholinesterase as a measure of roentgen effect does not seem appropriate.

The results of this study indicate that the inhibition of the cholinesterase following roentgen radiation is not dependent upon the liberation of histamine or potassium chloride.

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